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Cytology and Fertility of Advanced Populations of *Elymus lanceolatus* (Scribn. & Smith) Gould × *Elymus caninus* (L.) L. Hybrids

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ABSTRACT

Within the wheatgrasses and wildryes, amphiploids are frequently made as a means for introgressing desirable traits and restoring fertility in hybrids between diverse species. This study reports the cytology, fertility, and morphological characteristics of *Elymus lanceolatus* (Scribn. & Smith) Gould, *E. caninus* (L.) L., their F_1 hybrids, advanced generations (F_7 and F_8), and three generations of advanced amphiploid progenies (C_1, C_2, and C_3). Meiotic chromosome associations of *E. lanceolatus* and *E. caninus* are typical of allotetraploids. Chromosome pairing in the F_1 hybrids suggests a close relationship between the two parents. Bivalent associations most frequently observed in the F_7 and F_8 were 14 bivalents. After multiple generations of harvesting available seed each generation from 10 plants, an increase in meiotic regularity was observed in the advanced F generations. Aneuploidy in the amphiploids (C generation) was observed in the C_2 and C_3 generations with chromosome numbers ranging from 47 to 56. The C_1 generation had significantly fewer univalents per cell than the C_2 and C_3 generations. Combined across chromosome numbers, there was a significant decrease in the number of bivalents from 22.48 to 21.36 to 20.27 in each succeeding C generation, respectively. After seven generations of seed increase, pollen stainability increased from less than 1% in the F_1 hybrid to 87 and 85% in the F_7 and F_8 generations, respectively. Chromosome doubling significantly reduced pollen stainability in the C_1, C_2, and C_3 generations as compared to the parents and advanced F generations. Cluster analysis was able to separate the parents and the different hybrid populations.

Within the wheatgrasses and wildryes, amphiploidy is a mechanism to introgress desirable traits (genes) and restore fertility in hybrids between diverse species. Amphiploidy was used in the development of the SL-1 germplasm (Asay et al., 1991) that combined diploid bluebunch wheatgrass [*Pseudoroegneria spicata* (Pursh) A. Love] with tetraploid thickspike wheatgrass [*E. lanceolatus* (Scribn. & Smith) Gould] at the hexaploid ($2n = 6x = 42$) level. Colchicine induced tetraploids of diploid crested wheatgrass [*Agropyron cristatum* (L.) Gaertn.] and Russian wildrye [*Psathyrostachys juncea* (Fisch.) Nevski] were used to introgress diploid and tetraploid germplasm (Asay et al., 1985; unpublished data, 2004). Cytological instability and low fertility are commonly associated with newly formed amphiploids (Ashman and Boyle, 1955; Hill and Buckner, 1962). Dewey (1968) concluded that whether or not infertility, cytological instability, and lack of vegetative vigor are present in early generations, these obstacles must be overcome if developed amphiploids are to have an impact in developing new cultivars. Little is known regarding the effect of advanced amphiploid generations on meiotic stability and fertility within the wheatgrasses and wildryes.

Most of the perennial grass species in the tribe Triticeae are allopolyploids that originated from genome combinations of two or more species. *Elymus*, as circumscribed by Dewey (1984) and Löve (1984), is the largest genus in the tribe, with more than 125 species represented in most temperate and subarctic regions. About 75% of *Elymus* polyploid species are allotetraploids ($2n = 4x = 28$) that arose from hybridization among St-, H-, and Y-genome diploids ($2n = 14$). *Elymus lanceolatus* is a glaucus, stiff-leafed, rhizomatous, cross-pollinating (Jensen et al., 1990) grass of considerable economic importance on arid rangelands in the western USA. *Elymus caninus* is a green, lax-leafed, caespitose, self-pollinating (Jensen et al., 1990) species distributed in forest regions throughout Europe and eastward to Afghanistan (Hubbard, 1968). These species are allotetraploids. The St genome in *E. lanceolatus* and *E. caninus* originated from the new and old world *Pseudoroegneria* species, respectively (Stebbins and Snyder, 1956; Dewey, 1965). The H genome can be traced to a small-seeded *Hordeum* species (formerly *Critesion*; Dewey, 1984) inhabiting both the new and old worlds. Chromosome pairing in F_1 hybrids between *E. lanceolatus* (PI 233664) × *E. caninus* (PI 235438 and PI 252044) averaged 1.6 univalents + 13.1 bivalents + 0.03 trivalents + 0.03 quadrivalents per cell (Dewey, 1970), confirming that both species share the same basic genomes (StStHH).

The ecological and geographical differences between *E. lanceolatus* and *E. caninus* suggest that they evolved through independent hybridization events involving either diploid *Pseudoroegneria* species (StSt) and diploid *Hordeum* species (HH) or their autotetraploid counterparts (StStStSt; HHHH). F_1 hybrids between *E. lanceolatus* and *E. caninus* were reported to be mostly self-fertile with stainable pollen ranging from 5 to 25% and seed yield ranging from 2 to 600 seeds per plant under open-pollination (Dewey, 1970).

The induced amphiploid (C_0; $2n = 8x = 56$; StStStSt HHHH) between *E. lanceolatus* and *E. caninus* averaged 2.7 univalents + 16.9 bivalents + 1.3 trivalents + 3.9 quadrivalents per cell (Dewey, 1970). Increased meiotic irregularities resulted in a 50% reduction in hybrid fertility in the C_0 amphiploid (Dewey, 1970). The present study confirms the cytology and fertility of *E. lanceolatus*, *E. caninus*, F_1 hybrid, and amphiploid (C_0) as previously reported by Dewey (1970) and examines the cytology, fertility, and morphological characteristics of advanced generations (F_7 and F_8), and three generations of advanced amphiploid progenies (C_1, C_2, and C_3). The objectives of the study were to compare chromosome pairing, fertility, and morphology of *E. lanceolatus*, *E.*

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caninus, F_1 hybrids, and C_0 amphiploids with F_7 and F_8 hybrids and C_1, C_2, and C_3 amphiploids.

MATERIALS AND METHODS

Plant nomenclature follows the "genomic system of classification" (Dewey, 1984) and genome designations are after Wang et al. (1995). Development and chromosome pairing in the original parents, F_1 hybrids, and amphiploid hybrids (C_0) were reported by Dewey (1968, 1970). Details describing the origin of the parents and hybrid generation are reported in Dewey (1970). Each generation (F_1–F_8) was advanced by randomly compositing open-pollinated seed from a minimum of 10 plants per generation used to establish the next generation. Amphiploid generations were advanced in a similar fashion for C_0, C_1, C_2, and C_3.

Since fertility and morphology can be affected by the environment, cytological, fertility, and morphological data were taken on the parents and all hybrid populations in a common garden. Seedlings obtained from open-pollinated seed of *E. lanceolatus* (PI 233664), *E. caninus* (PI 252044), and their hybrid populations were established at the Utah State University Evans Research Farm, approximately 2 km south of Logan, UT (41°45' N, 111° 8' W, 1350 m above sea level). Soil at the site is a Nibley silty clay loam series (fine, mixed, active, mesic Aquic Argixerolls). The 40-yr (1951–1999) average annual precipitation at the site was 455 mm with about one-half occurring from May through October.

Cytological Samples and Squash Preparations

Spikes for cytological analysis were collected from *E. lanceolatus*; *E. caninus*; F_1, F_7, and F_8 generation hybrids; and amphiploid generations C_1, C_2, and C_3. The samples were fixed in Carnoy's fixative (absolute alcohol/chloroform/acetic acid, 6:3:1) for 24 to 48 h, transferred to 70% ethanol, and stored in a refrigerator at 4°C until analyzed. Squash preparations of the pollen mother cells were stained with an acetocarmine solution. Meiotic data were collected at metaphase I.

Pollen Stainability and Seed Set

Spikes for pollen stainability were collected at anthesis for accessions of *E. lanceolatus*, *E. caninus*; F_1, F_7, and F_8 generation hybrids; and amphiploid generations C_1, C_2, and C_3. The pollen grains were immersed in a I_2KI (iodine–potassium iodide) solution, which stains starch found in viable pollen grains black or dark gray. Aborted pollen grains are shrunken and light-amber colored in I_2KI. A minimum of 1000 pollen grains were scored as viable or inviable for each parent and hybrid generation. Seed set under open-pollination for *E. lanceolatus*, *E. caninus*, and their advanced hybrids was determined on 10 spikes from each plant harvested one month after anthesis. The spikes were hand threshed and seed counted to estimate plant fertility expressed as seeds per spike.

Morphological Traits

Morphological variations in the parents and the hybrid populations were measured on plant height (cm), flag leaf width (mm), flag leaf length (cm), leaf number, and internode number using 15 to 20 different plants of *E. lanceolatus*, *E. caninus*, and advanced generation hybrids. From each plant, morphological data were collected as the mean of five measurements. Principal components were derived using correlation matrices. Cluster analysis was performed using unweighted pair group mathematical average (UPGMA) algorithms on the distance matrices to provide a distance phenogram. The distance coefficient was defined as the average taxonomic distance computed by NT-SYS (Rohlf, 1992). All data were subjected to analysis of variance using GLM procedures as a fixed model. Mean separations were made on the basis of least significant differences (LSD) at the 0.05 probability level (SAS Institute, 1999).

RESULTS AND DISCUSSION

Cytology

Parents

Meiotic chromosome pairing associations in *E. lanceolatus* and *E. caninus* confirmed that both species are allotetraploids ($2n = 28$) (Table 1). Almost exclusive bivalent pairing (14 bivalents; Table 1) in *E. lanceolatus* (StStHH) (Fig. 1a) and *E. caninus* (StStHH) (Fig. 1b) is similar to that reported by Stebbins and Snyder (1956) and Dewey (1965, 1967, 1968, 1970) in the above taxa and other allotetraploid species within *Elymus*. Univalents and multivalents were rarely observed in the 182 cells of *E. lanceolatus* and 200 cells of *E. caninus* examined (Table 1).

F_1 Hybrids

As expected, all F_1 hybrids had chromosome numbers of $2n = 28$ and most chromosomes paired normally at metaphase I (Table 1). The most common meiotic configuration was 14 bivalents (Fig. 1c), which occurred in 116 of 200 cells (58%) interpreted. Another 18% of the metaphase I cells contained two univalents and 13 bivalents (Fig. 1d). The high proportion of ring-bivalents, usually 10 to 13 per cell and a c-value of 0.87 (mean arm-pairing frequency; Alonso and Kimber, 1981), supported previous conclusions (Dewey, 1970) that there are close homologies between the New World *E. lanceolatus* and the Old World *E. caninus* chromosomes. The remaining hybrid cells had various combinations of univalents, bivalents, and occasional multivalents.

F_7 to F_8 Generations

Meiosis was checked in 20 F_7 and 10 F_8 plants. All plants had a chromosome number of $2n = 28$. Meiosis was generally more regular in the F_7 and F_8 generations than in the F_1 (Table 1), where 96 and 85% of the metaphase I cells formed 14 bivalents (Fig. 1e), respectively. The occurrence of trivalents and quadrivalents in less than 2% of the metaphase I cells suggests the lack of any heterozygous interchange within advanced F generation hybrids of *E. lanceolatus* × *E. caninus*. In the F_7, the c-value of 0.95 approached that of the parental species (Table 1). An increase in meiotic regularity was observed in the advanced F generations.

Induced Amphiploids (C_1–C_3)

Four initial amphiploid plants (C_0) with a chromosome number of $2n = 8x = 56$ (octaploid) resulted from 35 F_1 vegetative tillers of *E. lanceolatus* × *E. caninus* hybrids treated with colchicine (Dewey, 1970). Based on the octoploid genomic composition in the C_0, StStSt StHHHH, unpaired chromosomes were observed in more than half of the metaphase I cells and multivalents consisting of three or four chromosomes were observed in all cells (Dewey, 1970). Dewey (1970) reported a range

Table 1. Chromosome pairing in *Elymus lanceolatus* (StStHH), *E. caninus* (StStHH), F_1 hybrids (StStHH), and hybrid derivatives.

			Chromosome associations (No. cell^{-1})										
				II				IV					
Species	No. plants	Chromosome no. ($2n$)	I	Ring	Rod	Total	III	Ring	Rod	Total	V+	No. cells	c-value§
E. lanceolatus	4	28	0.06†	12.22	1.72	13.94	0.005	0.005	0.005	0.01		182	0.94
			0–2‡	9–14	0–5	10–14	0–1	0–1	0–1	0–1			
E. caninus	4	28	–	13.51	0.49	14.00	–	–	–	–		200	0.98
			–	10–14	0–4	14.00	–	–	–	–			
E. lanceolatus × *E. caninus* F_1	4	28	0.68	10.42	2.84	13.26	0.09	0.3	0.12	0.14		200	0.87
			0–6	6–14	0–8	9–14	0–2	0–1	0–1	0–2			
E. lanceolatus × *E. caninus* F_7	20	28	0.05	12.86	1.10	13.96	–	0.004	–	0.004		500	0.95
			0–2	8–14	0–5	13–14	–	0–1	–	0–1			
E. lanceolatus × *E. caninus* F_8	10	28	0.32	11.60	2.16	13.76	0.008	0.004	0.02	0.024		250	0.91
			0–8	5–14	0–9	9–14	0–1	0–1	0–1	0–2			
E. lanceolatus × *E. caninus* C_1	10	56	0.94	20.29	2.20	22.49	1.2	0.72	0.84	1.56	0.07	250	0.91
			0–6	13–26	0–8	16–28	0–5	0–3	0–4	0–5	0–1		
E. lanceolatus × *E. caninus* C_2	8	56	1.31	19.55	1.96	21.50	1.37	0.79	1.04	1.83	0.05	200	0.9
			0–5	13–25	0–7	15–28	0–5	0–3	0–3	0–5	0–1		
E. lanceolatus × *E. caninus* C_2	1	55	0.84	19.80	1.60	21.40	0.80	1.32	0.88	2.20	0.04	25	0.91
			0–3	16–24	0–7	20–28	0–2	0–3	0–3	0–5	0–1		
E. lanceolatus × *E. caninus* C_2	1	54	1.20	16.88	3.36	20.24	2.28	0.64	1.12	1.76	0.08	25	0.86
			0–4	11–21	0–9	20–28	1–4	0–2	0–3	0–3	0–1		
E. lanceolatus × *E. caninus* C_3	5	56	1.09	19.48	2.25	21.73	1.48	0.74	0.98	1.71	0.03	125	0.90
			0–6	11–25	0–9	16–28	0–5	0–3	0–3	0–5	0–1		
E. lanceolatus × *E. caninus* C_3	1	55	1.00	20.56	1.84	22.40	1.24	0.60	0.72	1.32	0.04	25	0.90
			0–3	15–25	0–6	19–26	0–3	0–2	0–2	0–2	0–1		
E. lanceolatus × *E. caninus* C_3	3	54	1.53	18.03	2.00	20.03	1.72	0.63	1.12	1.75	0.05	75	0.85
			0–5	13–23	0–6	13–25	0–5	0–3	0–5	0–6	0–1		
E. lanceolatus × *E. caninus* C_3	1	47	6.16	9.88	1.76	11.64	5.04	0.12	0.44	0.56	0.04	25	0.60
			1–10	6–14	0–4	7–15	2–9	0–1	0–2	0–2	0–1		

† Configuration mean number of chromosomes.
‡ Configuration range of number of chromosomes.
§ c-value is defined as the mean arm-pairing frequency (Alonso and Kimber, 1981).

in chromosome numbers of 55 to 58 in the C_1 amphiploid between *E. lanceolatus* × *E. caninus*. However, he did not report on chromosome pairing in advanced amphiploids. In the present study, all C_1 plants were $2n = 56$. Aneuploidy in the amphiploids was observed in the C_2 and C_3 generations (Table 1) with chromosome numbers ranging from 47 to 56.

Because of complex pairing relationships found in octaploid amphiploids comprised of only two genomes, pairing relationships were subjected to analysis of variance to identify possible trends in chromosome pairing as a result of advanced generations and varying chromosome numbers. Univalents were observed in 56% of the C_1 cells at metaphase I and multivalents (Fig. 1f) consisting of three to seven chromosomes formed in 94% of the cells. C_1 hybrids had significantly ($P < 0.05$) fewer univalents per cell than C_2 and C_3 hybrid generations. Complete pairing, 28 bivalents, were observed in 9 of the 250 cells examined. Combined across chromosome numbers, there was a significant decrease ($P < 0.05$) in the

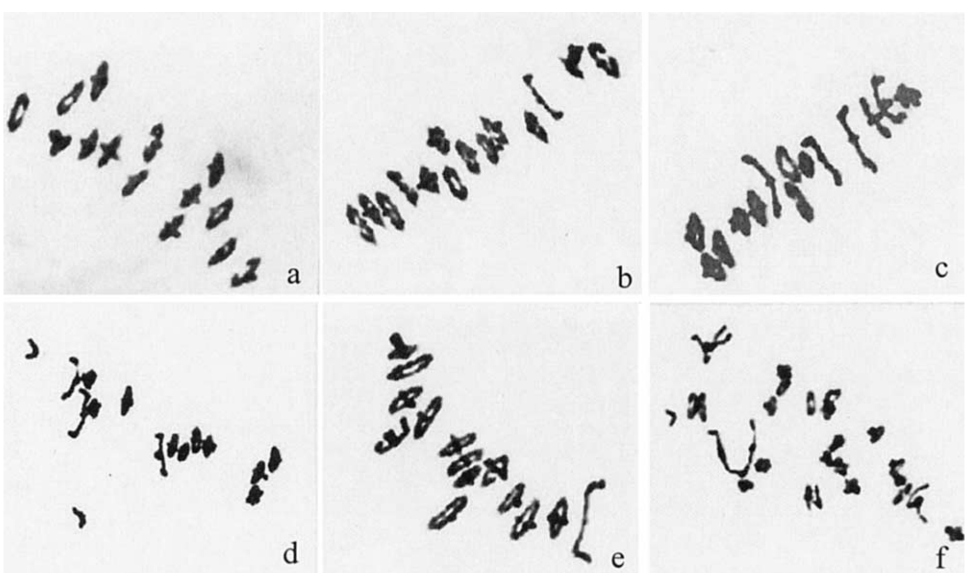

Fig. 1. Meiotic chromosome associations for *Elymus lanceolatus*, *E. caninus*, and hybrids. (a) *E. lanceolatus*-14 II, (b) *E. caninus*-14 II, (c) *E. lanceolatus* × *E. caninus* F_1 hybrid-14 II, (d) *E. lanceolatus* × *E. caninus* F_1 hybrid-2 I + 13, (e) *E. lanceolatus* × *E. caninus* F_7 hybrid-14 II, and (f) *E. lanceolatus* × *E. caninus* C_3 hybrid-1 I + 18 II + 1 III + 4 IV.

number of bivalents observed from 22.48 to 21.36 to 20.27 in each succeeding C_1, C_2, and C_3 generation, respectively. Selection for seed yield in the C_1 resulted in an increase of nearly six bivalents per cell over those reported in the C_0 generation (Dewey, 1970); however, in subsequent generations, the mean bivalent frequency declined (Table 1). The increase in univalents per cell and decrease in bivalent formation appears to be associated with increased aneuploidy observed in C_2 and C_3, particularly associated with the $2n = 47$ C_3 plant. In C_2 and C_3 generations, 56 and 55 chromosome plants had significantly ($P < 0.05$) more bivalents per cell than 54 and 47 chromosome plants (Table 1).

In the C_1, 34% of the cells lacked trivalents compared to 21 and 26% in the C_2 and C_3 generations, respectively. Combined across chromosome numbers, generation C_3 has significantly more trivalents ($P < 0.05$) than C_2 or C_1. By excluding the $2n = 47$ C_3 plant, which averaged 5.04 trivalents per cell, from the analysis, there was no significant difference from C_2 to C_3 in trivalent frequency. Generation C_1 has the lowest trivalent frequency ($P < 0.05$), suggesting possible chromosome instability within aneuploids in advanced generations after chromosome doubling.

Over 80% of the metaphase I cells in generations C_1, C_2, and C_3 exhibited between one and five quadrivalents. The most frequently observed association was one quadrivalent per cell, which occurred in 37% of the cells. There was no significant difference in quadrivalent frequency among the aneuploids within C_2 and C_3, excluding the $2n = 47$ plant. The 47-chromosome plant had significantly ($P < 0.05$) fewer quadrivalents than the mean quadrivalent frequency in C_2 and C_3 (Table 1). The origin of the 47-chromosome plant is uncertain. If chromosomes within a genome were lost at random from the C_2 to C_3, one would not expect to observe a loss in quadrivalents and an increase in univalents (Table 1). However, the occurrence of six univalents per cell, with a range of 1 to 10 univalents, suggests that the 47-chromosome plant may have originated from a cross with a parent whose genomic formula is either St_ or H_. The latter is unlikely because there are no reports of the H genome being involved in polyploid evolution with anything but the St and possibly Y genomes. The known genome would pair as triploids with chromosomes from the amphiploid accounting for the increase (5.04) in trivalent frequencies. The unknown genome would then be left as univalents.

Fertility

Percentage stainable pollen and seed set under open pollination was higher in the self-pollinated *E. caninus* than in the cross-pollinated *E. lanceolatus* (Table 2). Although chromosome pairing appeared regular, the F_1 hybrids had less than 1% stainable pollen and set less than 1 seed per spike under open pollination, suggesting a genic barrier between these geographically isolated entities. After seven generations, where seed was harvested and grown-out, pollen stainability increased to 87 and 85% in the F_7 and F_8 generations, respectively. Seed set increased from less than 1 seed per spike to 64.6 and 68.5 seeds per spike in the F_7 and F_8, respectively. This demonstrates that with limited seed production in the F_1, that by harvesting seed and advancing the generation, fertility can be restored in this hybrid combination.

Chromosome doubling significantly reduced pollen stainability ($P < 0.05$) in the C_1, C_2, and C_3 generations from that of the parents and advanced F generations (Table 2). Ten C_1's produced 20 to 80% stainable pollen and averaged 50.7%. The average stainable pollen in the C_1 generation was similar to the 50.9% reported by Dewey (1970) in C_1 hybrids of *E. lanceolatus* × *E. caninus*. Despite the reduced pollen stainability in the C_1, seed yield was only slightly lower when compared to the advanced F generations (Table 2). Seed yield in the C_1 ranged from 27 to 139 seeds per spike. The reduction in mean number of darkly stained pollen grains and seed yield among the C_2 and C_3 plants might be associated with the increased frequency of aneuploidy with each succeeding generation. The C_3 generation, which had the highest incidence of aneuploidy, also had the greatest amount of variability in stainable pollen, ranging from 3 to 72%. Consequently the number of seeds per spike declined from 112 seeds in the C_1 to 51 and 29 in C_2 and C_3, respectively. Reduced seed set in the amphiploid generations may be attributed to the reduction in viable pollen as a result of increased aneuploidy.

Table 2. Fertility characteristics used to evaluate variation in *Elymus lanceolatus*, *E. caninus*, F_1 hybrids, and hybrid derivatives.

Species	% Stainable pollen	No. plants	Mean† no. seeds spike^{-1}	No. plants
E. lanceolatus	77.0	10	13.8	9
E. caninus	92.4	10	92.6	10
E. lanceolatus × *E. caninus* F_1	0.2	5	0.1	5
E. lanceolatus × *E. caninus* F_7	87.1	10	64.6	20
E. lanceolatus × *E. caninus* F_8	84.5	10	68.5	19
E. lanceolatus × *E. caninus* C_1	50.7	10	66.9	10
E. lanceolatus × *E. caninus* C_2	44.3	10	54.6	10
E. lanceolatus × *E. caninus* C_3	36.6	10	44.1	9
LSD (0.05)	11.1		16.5	

† Mean based on 10 spikes plant^{-1}.

Table 3. Morphological characteristics† used to evaluate variation in *Elymus lanceolatus*, *E. caninus*, F_1 hybrids, and hybrid derivatives.

Species	Plant height	Leaf width	Leaf length	No. leaves	No. internodes
	cm	mm	cm		
E. lanceolatus	64 ± 9.9	4.6 ± 0.7	15.3 ± 1.4	2.3 ± 0.4	2.8 ± 0.35
E. caninus	87 ± 5.2	9.9 ± 1.0	19.7 ± 1.5	3.5 ± 0.3	4.5 ± 0.33
E. lanceolatus × *E. caninus* F_7	83 ± 10.6	6 ± 1.0	17.7 ± 2.7	2.7 ± 0.4	3.4 ± 0.35
E. lanceolatus × *E. caninus* F_8	88 ± 8.0	6.6 ± 0.9	17.6 ± 2.1	3.0 ± 0.4	3.3 ± 0.40
E. lanceolatus × *E. caninus* C_1	86 ± 8.6	8.8 ± 1.1	20.4 ± 2.5	3.3 ± 0.3	4.2 ± 0.28
E. lanceolatus × *E. caninus* C_2	80 ± 6.7	8.2 ± 1.0	19.7 ± 1.8	3.4 ± 0.3	4.3 ± 0.27
E. lanceolatus × *E. caninus* C_3	84 ± 9.1	8.8 ± 0.8	19.8 ± 3.9	3.4 ± 0.3	4.2 ± 0.30
LSD (0.05)	5.2	0.6	1.5	0.19	0.21

† Mean ± SE.

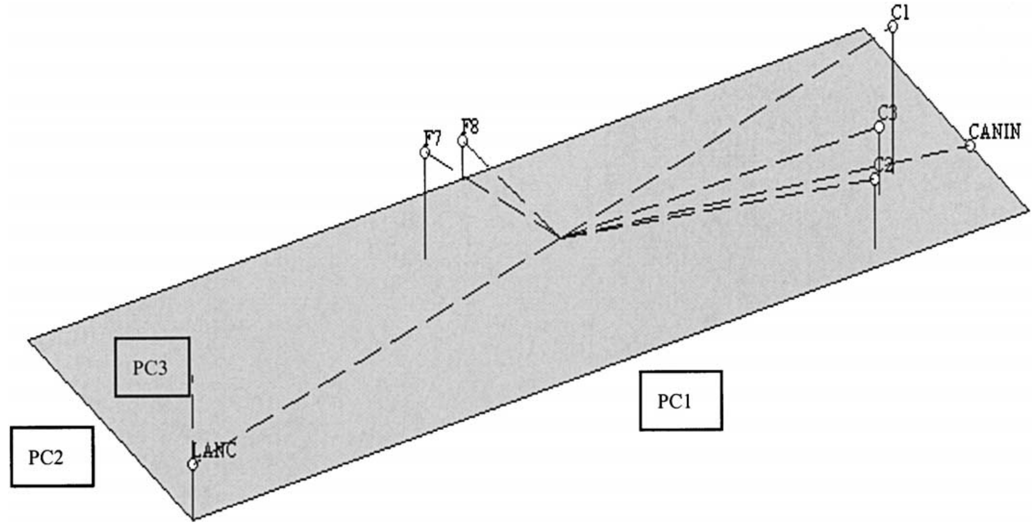

Fig. 2. Cluster analysis of five morphological characters plant height (cm), flag leaf width (mm), flag leaf length (cm), leaf number, and internode number for *Elymus lanceolatus* (LANC), *E. caninus* (CANIN), F_1 hybrids, hybrid derivatives (F_7, F_8), and amphiploid populations (C_1, C_2, and C_3).

Morphology

Based on five morphological characters including [plant height (cm), leaf width (mm), leaf length (cm), number of leaves per culm, and number of internodes per culm (Table 3)], principal components accounted for 99% of the variation in the first three axes. Within the first component (PC1), which accounted for 88% of the variation, leaf width, leaf length, leaf number, and internode number all had factor weightings > 0.90. Components 2 and 3 were much less diagnostic, with plant height (0.64) having the highest weighting in component 2. Cluster analysis of the three principal components (Fig. 2) isolated *E. lanceolatus* based on its shorter plant height and leaf length, narrower leaves, and fewer leaves and internodes per culm. Despite having wider leaves than C_1, C_2, and C_3 (Table 3), *E. caninus* grouped closer to the C generations based on similar plant height, leaf length, and leaf and internode number. The F generation hybrids grouped together based on their intermediate leaf length (Table 3).

Seed set data in the F_7 and F_8 generations showed that fertility can be restored in F_1 hybrids between *E. lanceolatus* and *E. caninus* that exhibit regular chromosome pairing at metaphase I and have some, albeit limited, fertility. Under these circumstances, reduced fertility likely results from chromosomal differences too small to interfere with pairing and nonhomologous gene recombinations. Unequal disjunction of chromosomes at anaphase I is often associated with reduced fertility in amphiploid hybrids where an increase in multivalents and aneuploidy are observed. It also may be responsible for increased chromosome number in the amphiploid. Fertility declined in each subsequent generation in the amphiploid hybrid between *E. lanceolatus* × *E. caninus.* It is unlikely that fertility could be restored in this amphiploid with additional generation advances.

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